Supplementary Information for

Development of 4-Oxime-1,8-Naphthalimide as A Bioorthogonal Turn-on Probe for Fluorogenic Protein Labeling

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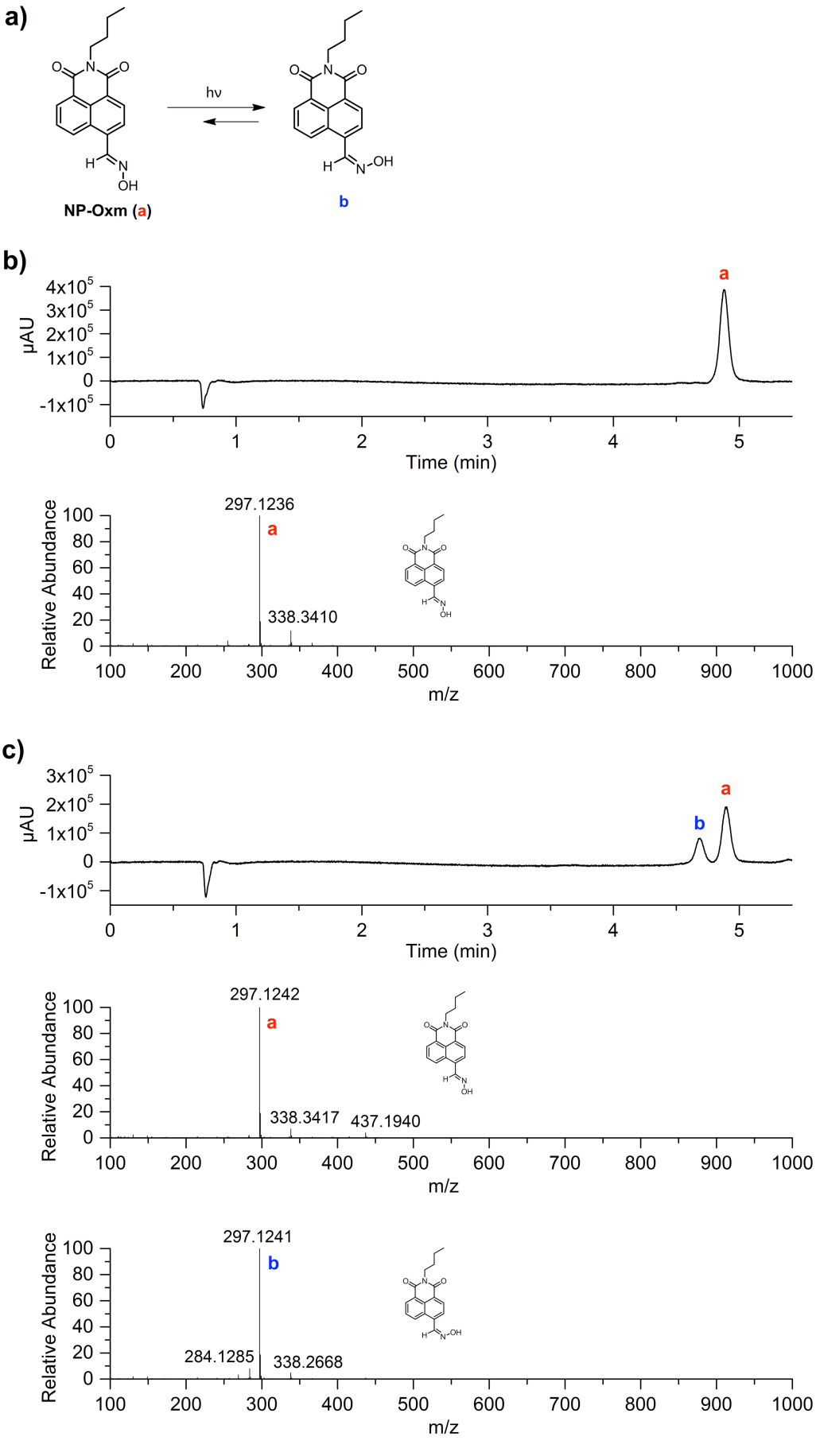
a)
hν
NP-Oxm (a)
b
b)
a
μAU
Time (min)
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338.3410
Relative Abundance
m/z
c)
a
b
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338.3417
437.1940
297.1241
284.1285
338.2668

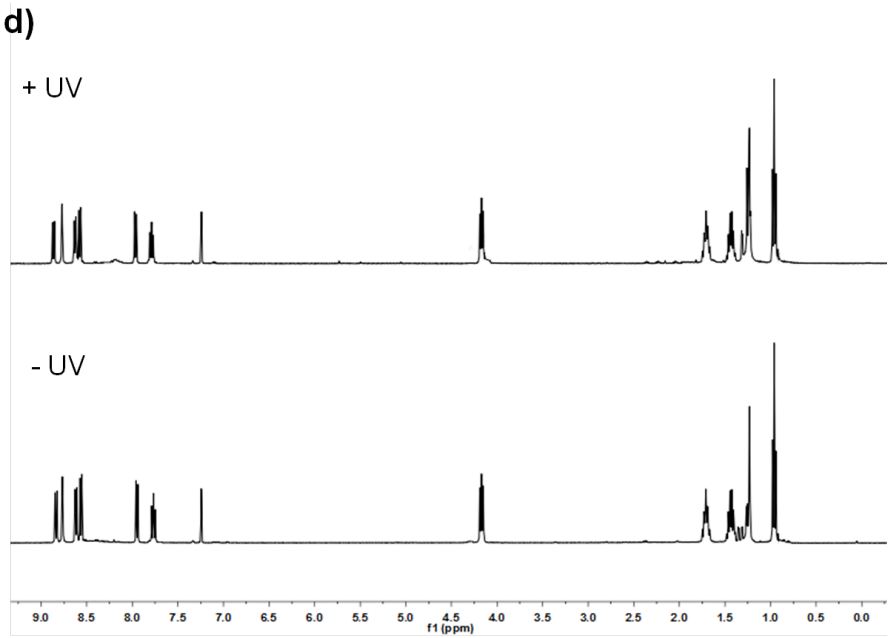
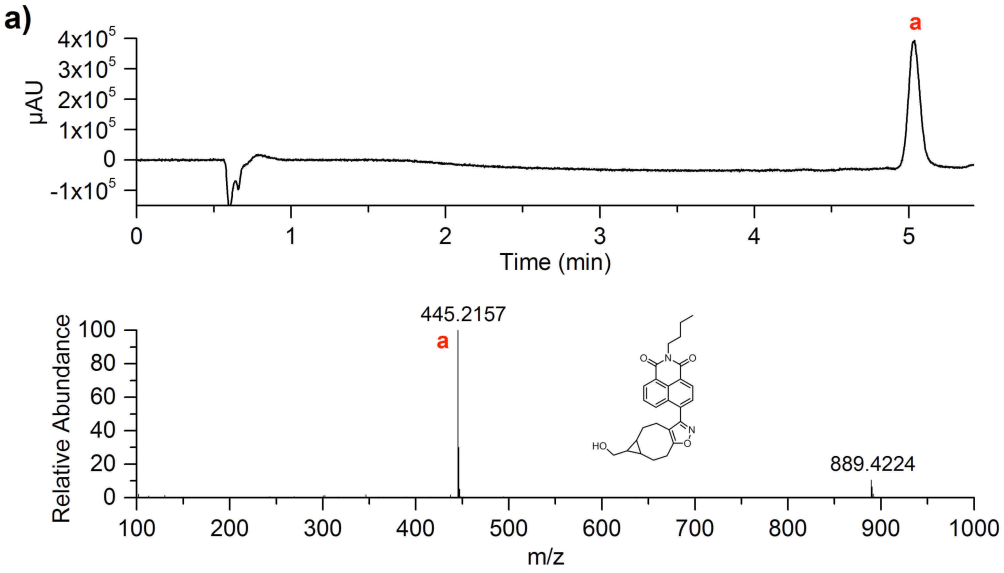


Fig S1. Photostability study of **NP-Oxm**. (a) E/Z isomerization of **NP-Oxm** upon UV irradiation. (b) LC/MS of **NP-Oxm**. (c) LC/MS of **NP-Oxm** upon 365 nm UV irradiation for 10 min. (d) ^{1}HNMR of **NP-Oxm** with (top) or without (bottom) 365 nm UV irradiation.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Time (min)
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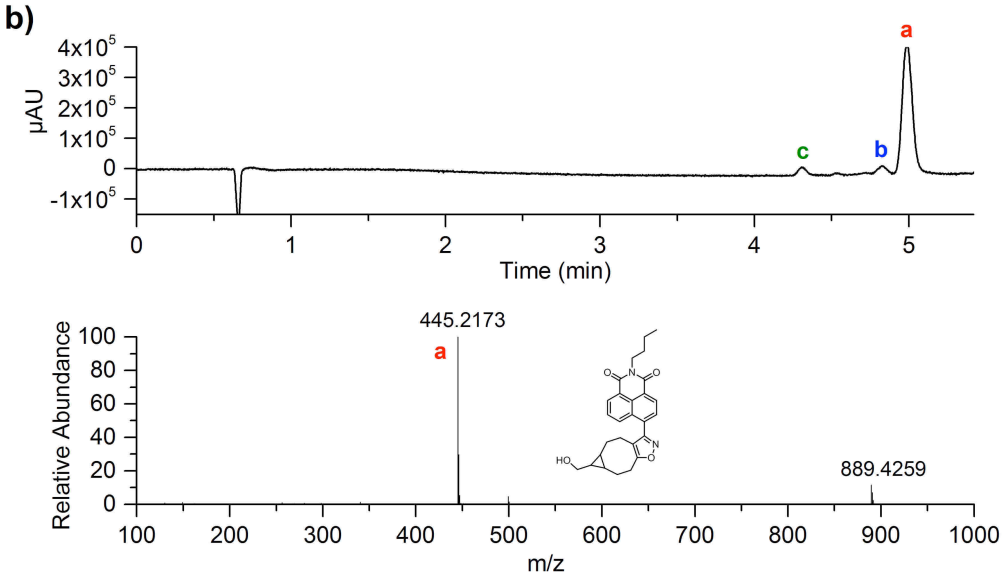
m/z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Time (min)
a
c
b
Relative Abundance
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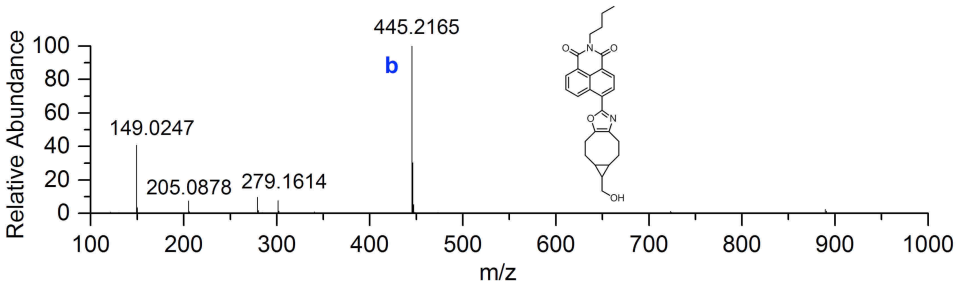
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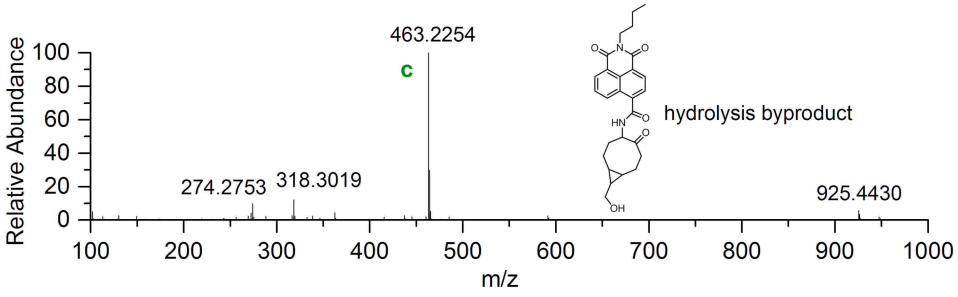
m/z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m/z

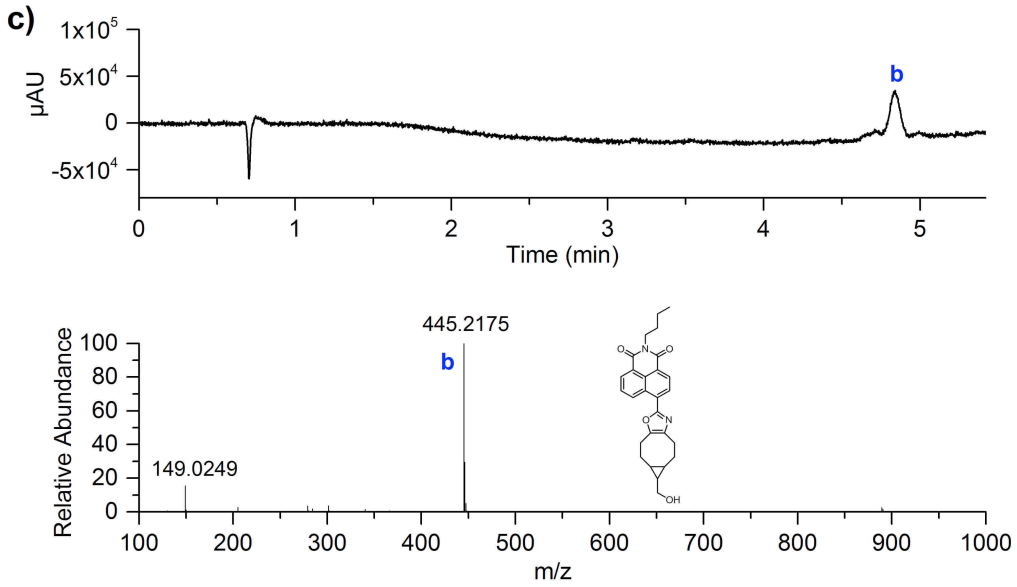


Fig S2. LC-MS characterization of isoxazole-oxazole photoisomerization. (a) LC-MS of **NP-BCN**. (b) LC/MS of **NP-BCN** upon 365 nm UV irradiation for 2 min. (c) LC-MS of purified 4-oxazolyl-1,8-naphthalimide.

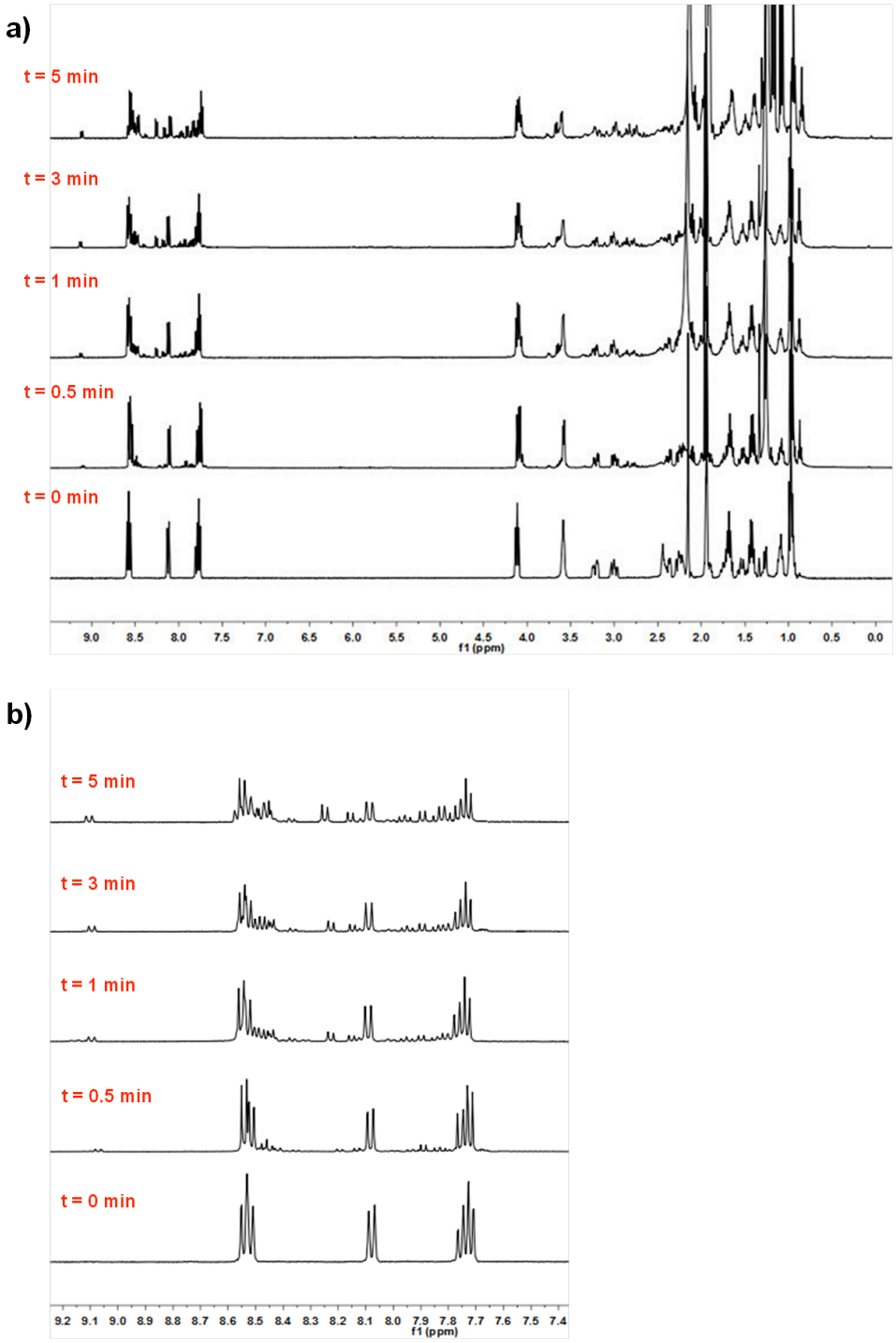
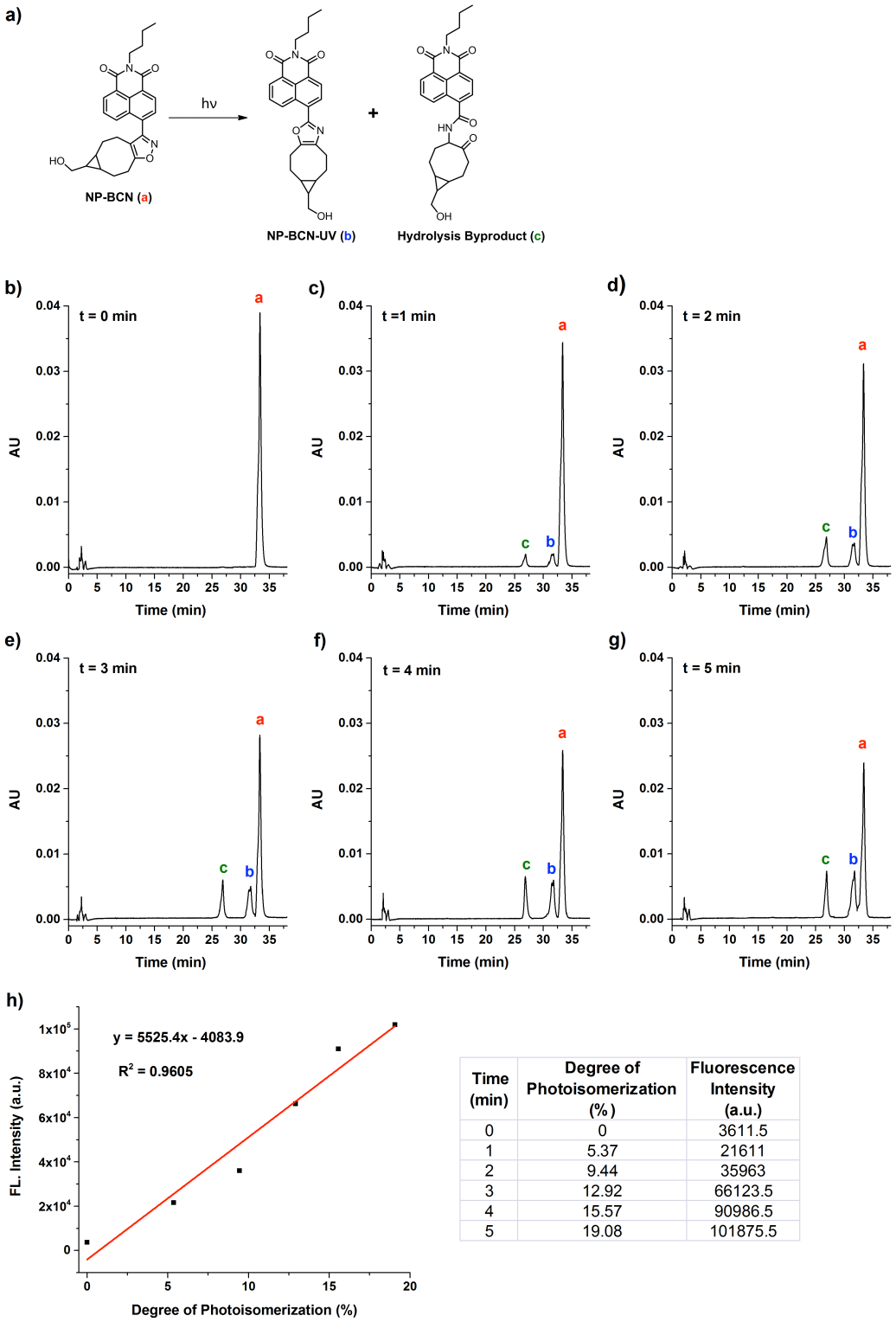


Fig S3. ^{1}HNMR of **NP-BCN** upon 365 nm UV irradiation for 0, 0.5, 1, 3, 5 min. (a) full ^{1}HNMR spectra. (b) ^{1}HNMR spectra of aromatic rings.



Time (min)	Degree of Photoisomerization (%)	Fluorescence Intensity (a.u.)
0	0	3611.5
1	5.37	21611
2	9.44	35963
3	12.92	66123.5
4	15.57	90986.5
5	19.08	101875.5

Fig S4. (a) Photoisomerization of **NP-BCN** to 4-oxazolyl-naphthalimide (**NP-BCN-UV**), along with the generation of hydrolysis byproduct. (b-g) HPLC trace of **NP-BCN** upon 365 nm UV irradiation for 0 min (b), 1 min (c), 2 min (d), 3 min (e), 4 min (f), 5 min (g). (h) Correlation of fluorescence intensity with the degree of photoisomerization of **NP-BCN**. The degree of photoisomerization was quantified by calculating the proportion of isomerization product (**NP-BCN-UV**) among three species (**NP-BCN**, **NP-BCN-UV**, and hydrolysis byproduct) based on HPLC spectra.

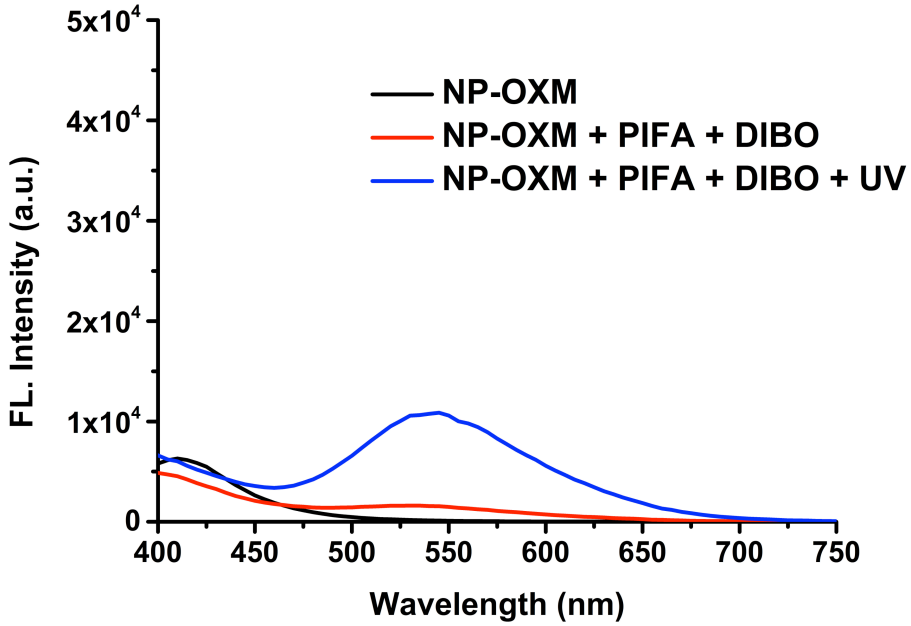


Fig S5. *In situ* fluorescence emission spectra change of 10 μM **NP-Oxm** in CH_3CN upon addition of PIFA, DIBO and photoirradiated with 365 nm UV for 10 min (λ_{exc} = 320 nm).

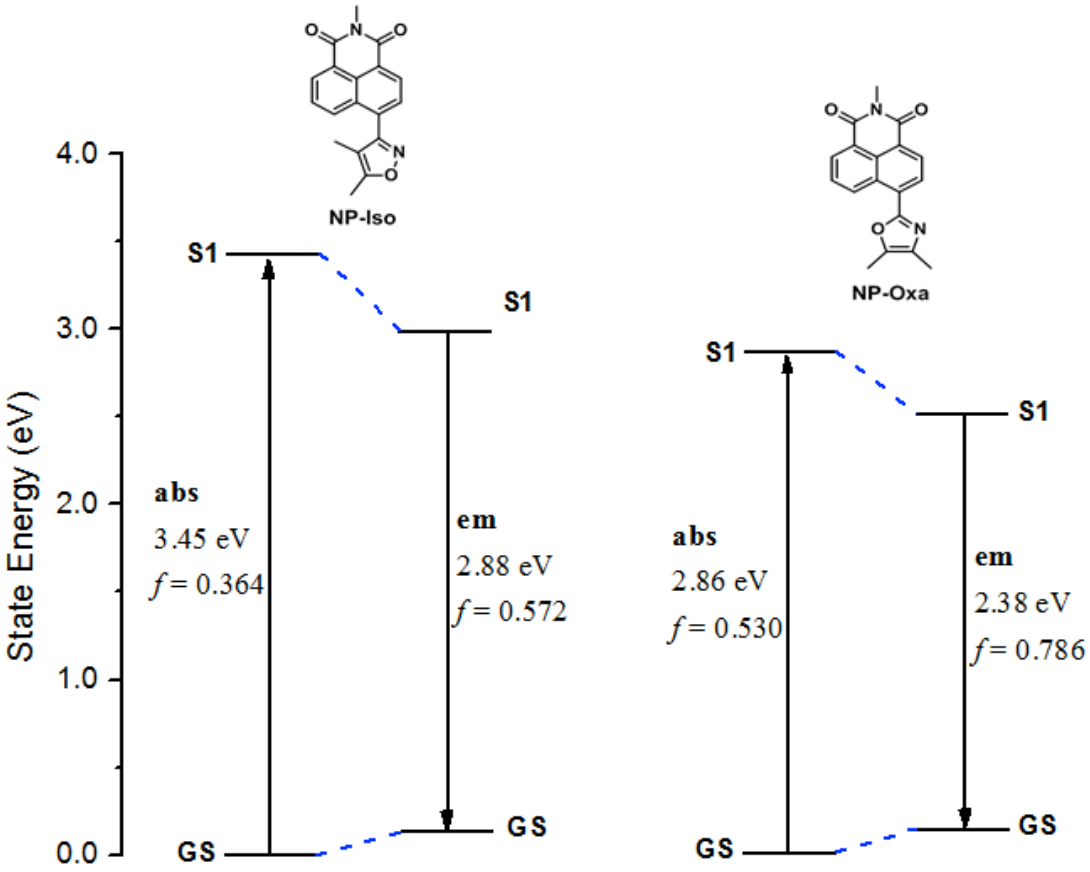


Fig S6. Energy diagrams of **NP-Iso** and **NP-Oxa** with their oscillator strength values.

Table S1. Cartesian atomic coordinates for the geometry-optimized structure of **NP-Iso** (B3LYP/6-311G(d), PCM with acetonitrile as solvent)

Atom	X (Å)	Y (Å)	Z (Å)
C	-0.574426	-2.040514	-0.355514
C	-1.559557	-1.089023	-0.187347
C	-1.207915	0.273882	-0.039002
C	0.165599	0.670611	-0.070275
C	0.777158	-1.665274	-0.385292
H	-0.856017	-3.080006	-0.470303
C	-2.22119	1.247904	0.144427
C	0.464584	2.04694	0.110645
H	1.528432	-2.430748	-0.536693
C	-0.537606	2.974542	0.292831
C	-1.885924	2.578576	0.305192
H	1.496572	2.368735	0.092292
H	-0.285912	4.019957	0.430337
H	-2.675227	3.306972	0.445304
C	-3.650808	0.857801	0.17071
C	-2.979307	-1.507532	-0.163034
O	-3.328506	-2.671235	-0.288602
O	-4.547386	1.672718	0.322011
N	-3.937003	-0.505404	0.012283
C	-5.339298	-0.93947	0.03095
H	-5.588677	-1.429307	-0.910044
H	-5.498814	-1.645639	0.845506
C	1.164607	-0.341346	-0.255931
C	2.609071	-0.024061	-0.310241
N	3.055941	0.939009	-1.088523
C	4.785891	0.001506	-0.015211
C	3.674198	-0.667646	0.406141
O	4.434779	0.957959	-0.906367
C	6.233688	-0.111082	0.301016
H	6.798317	-0.456074	-0.56984
H	6.643393	0.857001	0.599634
H	6.395839	-0.820143	1.112274
C	3.584161	-1.782767	1.402617
H	2.69351	-1.68438	2.026769
H	3.536772	-2.763687	0.920711
H	4.452026	-1.788853	2.064259
H	-5.961449	-0.062868	0.171037

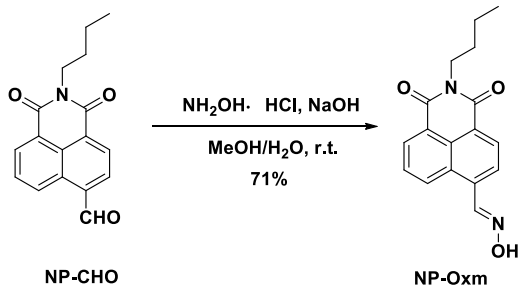
Table S2. Cartesian atomic coordinates for the geometry-optimized structure of **NP-Oxa** (B3LYP/6-311G(d), PCM with acetonitrile as solvent)

Atom	X (Å)	Y (Å)	Z (Å)
C	-0.690863	-2.101994	0.00017
C	-1.666724	-1.124474	0.000103
C	-1.291041	0.240807	-0.000002
C	0.090307	0.616722	-0.000005
C	0.663937	-1.756036	0.000141
H	-0.98575	-3.144016	0.000253
C	-2.295864	1.240937	-0.000113
C	0.397161	2.002927	-0.000125
H	1.4049	-2.544029	0.000194
C	-0.599837	2.955422	-0.000236
C	-1.952068	2.57885	-0.000231
H	1.43459	2.303763	-0.000097
H	-0.336942	4.007309	-0.000333
H	-2.737452	3.324885	-0.000321
C	-3.72845	0.867628	-0.000108
C	-3.091241	-1.527931	0.00013
O	-3.437422	-2.699605	0.000252
O	-4.62819	1.693999	-0.000204
N	-4.037392	-0.495909	0.000026
C	-5.463581	-0.84334	0.000065
H	-5.546532	-1.924198	0.000075
H	-5.94666	-0.430614	0.885459
C	1.080888	-0.428474	0.000066
C	2.51926	-0.1669	0.000055
C	4.525258	0.606388	0.000259
N	3.182071	0.954374	0.000433
O	3.343836	-1.254714	-0.000356
C	5.597476	1.644701	0.000607
C	4.621858	-0.753373	-0.000219
C	5.735281	-1.733061	-0.000646
H	5.517045	2.287512	0.881798
H	5.516872	2.288276	-0.880006
H	5.695536	-2.378585	0.881738
H	5.6955	-2.377858	-0.883564
H	6.591369	1.19574	0.00032
H	6.695186	-1.217056	-0.000463
H	-5.946736	-0.430607	-0.885276

General Information

Solvents and chemicals were purchased from commercial sources and used directly without further purification. ^{1}H NMR spectra were recorded on a Varian 400 MHz NMR spectrometer. ^{13}C NMR spectra were recorded on a Varian 500 MHz NMR spectrometer. Chemical shifts are referenced to the residual solvent peak and reported as δ units in ppm (in NMR description, s = singlet, d = doublet, t = triplet, q = quartet and m = multiple), and all coupling constant (J) values are given in hertz. ESI-HRMS data were measured on Thermo LCQ Deca XP Max mass spectrometer. Silica gel flash column chromatography was performed on Biotage Isolera one. Fluorescence emission spectra and full wavelength absorption spectra were recorded on Tecan Spark™ 10M Multimode Microplate Reader.

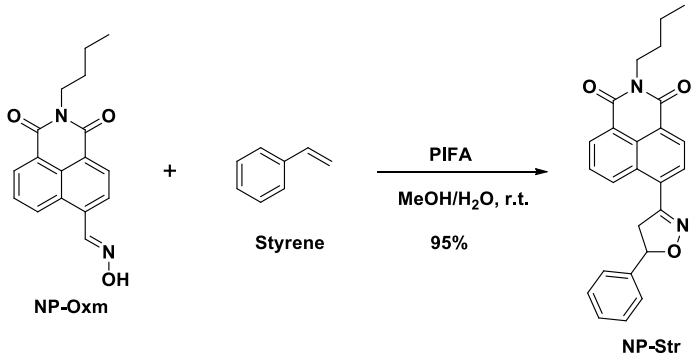
4-Formyl-1,8-naphthalimide (**NP-CHO**) was synthesized according to the literature procedure.[1]



NP-CHO NP-Oxm

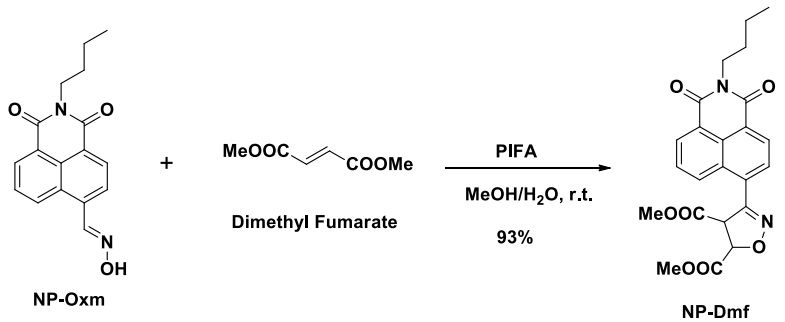
Synthesis of (*E*)-2-butyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-6-carbaldehyde oxime (NP-Oxm)[2]

To a solution of **NP-CHO** (510 mg, 1.81 mmol) in MeOH/H_2O (10 mL, 1:1) was added NH_2OH • HCl (132 mg, 1.9 mmol) and NaOH (76 mg, 1.9 mmol) at RT. After 30 min stirring, MeOH was evaporated and the resulting residue was extracted with EtOAc (3×8 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc=4:1) to afford the titled compound as yellow solid (381 mg, 71% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, J = 8.6 Hz, 1H), 8.78 (s, 1H), 8.63 (d, J = 7.2 Hz, 1H), 8.58 (d, J = 7.6 Hz, 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.78 (dd, J = 8.6, 7.2 Hz, 1H), 4.24 – 4.12 (m, 2H), 1.81 – 1.64 (m, 2H), 1.52 – 1.40 (m, 2H), 0.98 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.32, 164.02, 148.72, 134.25, 131.61, 131.12, 130.80, 129.10, 128.77, 127.88, 127.55, 123.65, 123.18, 40.62, 30.36, 20.59, 14.07; HRMS (ESI) calcd for $C_{17}H_{17}N_2O_3$ 297.1234 [M+H$^+$], found 297.1234.



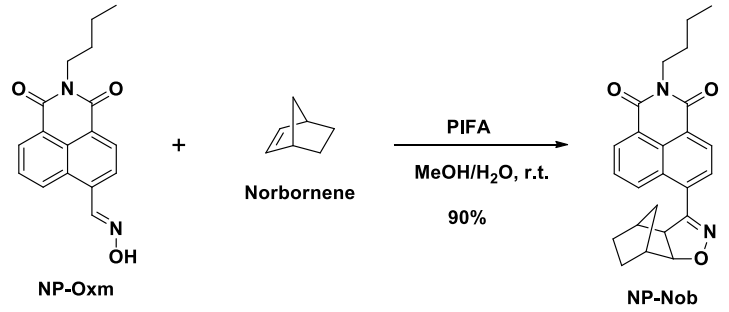
Synthesis of 2-Butyl-6-(5-phenyl-4,5-dihydroisoxazol-3-yl)-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (NP-Str)[2, 3]

To a solution of **NP-Oxm** (50 mg, 0.17 mmol) and PIFA (94 mg, 0.22 mmol) in MeOH/H_2O (6 mL, 5:1) was added styrene (35 mg, 0.34 mmol).The reaction mixture was stirred for 5 min at rt, then extracted with EtOAc (3 x 3 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 3:1) to afford the titled compound as yellow solid (64 mg, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.48 (d, *J* = 8.6 Hz, 1H), 8.63 (d, *J* = 7.3 Hz, 1H), 8.54 (d, *J* = 7.7 Hz, 1H), 7.83 (dd, *J* = 8.7, 7.3 Hz, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.50 – 7.32 (m, 5H), 5.84 (dd, *J* = 11.0, 8.4 Hz, 1H), 4.26 – 4.13 (m, 2H), 4.02 (dd, *J* = 16.5, 11.1 Hz, 1H), 3.60 (dd, *J* = 16.5, 8.4 Hz, 1H), 1.79 – 1.66 (m, 2H), 1.53 – 1.40 (m, 2H), 0.98 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.19, 163.77, 156.15, 140.41, 134.10, 132.25, 131.73, 130.31, 129.17, 129.04, 128.90, 128.79, 128.42, 127.98, 126.10, 124.00, 122.96, 82.53, 45.32, 40.58, 30.37, 20.60, 14.08; HRMS (ESI) calcd for $C_{25}H_{23}N_2O_3$ 399.1703 [M+H$^+$], found 399.1700.



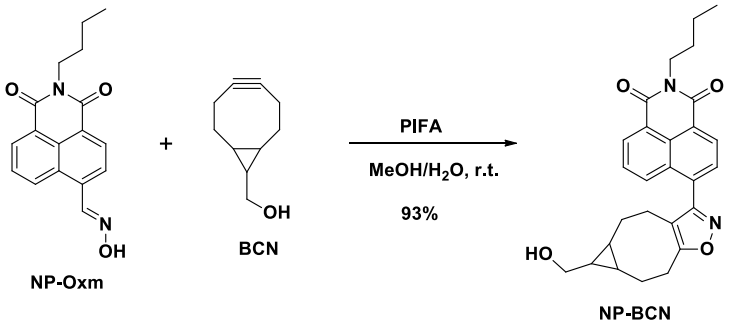
Synthesis of Dimethyl 3-(2-butyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin -6-yl)-4,5-dihydroisoxazole-4,5-dicarboxylate (NP-Dmf)

NP-Dmf was synthesized using the same procedure as **NP-Str** with 93% Yield. Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (d, *J* = 8.7 Hz, 1H), 8.65 (d, *J* = 7.3 Hz, 1H), 8.60 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.89 – 7.80 (m, 2H), 5.59 (d, *J* = 5.4 Hz, 1H), 5.14 (d, *J* = 5.4 Hz, 1H), 4.23 – 4.13 (m, 2H), 3.90 (s, 3H), 3.63 (s, 3H), 1.76 – 1.65 (m, 2H), 1.50 – 1.39 (m, 2H), 0.98 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.05, 167.92, 164.08, 163.69, 153.55, 133.12, 131.93, 130.51, 130.12, 129.56, 128.88, 128.58, 128.56, 124.58, 123.09, 81.85, 58.46, 53.73, 40.60, 30.36, 20.58, 14.06; HRMS (ESI) calcd for $C_{23}H_{23}N_2O_7$ 439.1500 [M+H$^+$], found 439.1496.



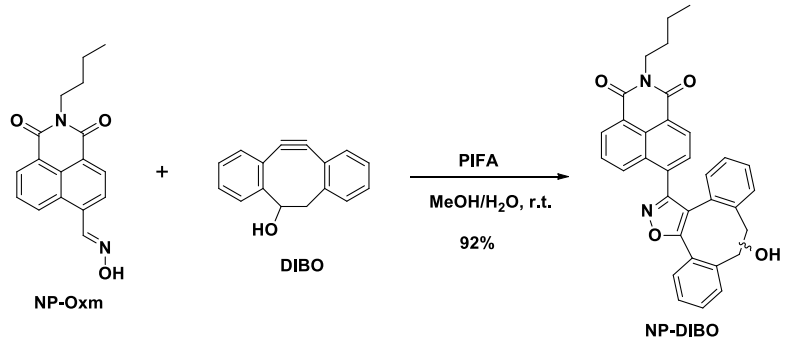
Synthesis of 2-Butyl-6-((3a*S*,4*S*,7*R*,7a*S*)-3a,4,5,6,7,7a-hexahydro-4,7-methanobenzo[*d*]isoxazol-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (NP-Nob)

NP-Nob was synthesized using the same procedure as **NP-Str** with 90% Yield. Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (d, *J* = 8.7 Hz, 1H), 8.62 (d, *J* = 7.3 Hz, 1H), 8.59 (d, *J* = 7.7 Hz, 1H), 7.85 – 7.76 (m, 2H), 4.73 (d, *J* = 8.4 Hz, 1H), 4.24 – 4.12 (m, 2H), 3.78 (d, *J* = 8.4 Hz, 1H), 2.73 (s, 1H), 2.40 (s, 1H), 1.76 – 1.66(m, 2H), 1.63 – 1.57 (m, 2H), 1.50 – 1.32 (m, 4H), 1.28 – 1.22 (m, 2H), 0.97 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.25, 163.86, 157.14, 134.06, 132.62, 131.68, 130.43, 129.71, 129.02, 128.21, 127.69, 123.68, 122.97, 87.69, 59.24, 43.51, 40.54, 39.61, 32.81, 30.37, 27.53, 22.99, 20.59, 14.07; HRMS (ESI) calcd for $C_{24}H_{25}N_2O_3$ 389.1860 [M+H$^+$], found 389.1867.



Synthesis of 2-Butyl-6-(6-(hydroxymethyl)-5,5a,6,6a,7,8-hexahydro-4*H*-cyclopropa[5,6]cycloocta[1,2-*d*]isoxazol-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (NP-BCN)

NP-BCN was synthesized using the same procedure as **NP-Str** with 93% yield. Light yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 – 8.61 (m, 2H), 8.13 (d, *J* = 8.4 Hz, 1H), 7.81 – 7.68 (m, 2H), 4.27 – 4.15 (m, 2H), 3.78 – 3.69 (m, 2H), 3.34 – 3.23(m, 1H), 3.11 – 2.98 (m, 1H), 2.48 – 2.38 (m, 1H), 2.38 – 2.25 (m, 2H), 2.09 – 1.97 (m, 1H), 1.80 – 1.66 (m, 2H), 1.50 – 1.40 (m, 2H), 1.31 – 1.13 (m, 4H), 1.08 – 0.95 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.32, 164.23, 164.04, 134.02, 132.09, 131.75, 131.15, 130.64, 130.55, 128.96, 128.57, 127.76, 123.86, 123.18, 114.46, 59.92, 40.59, 30.40, 27.16, 23.20, 22.91, 21.61, 21.38, 20.60, 19.98, 19.75,14.07; HRMS (ESI) calcd for $C_{27}H_{29}N_2O_4$ 445.2122 [M+H$^+$], found 445.2127.



Synthesis of 2-Butyl-6-(9/8-hydroxy-8,9-dihydrodibenzo[3,4:7,8]cycloocta[1,2-*d*]isoxazol-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (NP-DIBO)

NP-DIBO was synthesized using the same procedure as **NP-Str** with 92% yield. Light yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 – 8.38 (m, 3H), 7.84 – 7.60 (m, 4H), 7.48 – 7.30 (m, 3H), 7.22 – 7.13 (m, 1H), 6.88 – 6.79 (m, 1H), 6.58 – 6.50 (m, 1H), 5.66 – 5.24 (m, 1H), 4.23 – 4.12 (m, 2H), 3.78 – 3.52 (m, 1H), 3.51 – 3.38 (m, 1H), 1.77 – 1.67 (m, 2H), 1.50 – 1.40 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.20, 164.00, 159.88, 136.50, 132.49, 132.33, 132.10, 131.79, 131.77, 130.86, 130.46, 130.38, 130.12, 129.82, 129.71, 128.96, 128.78, 128.71, 128.68, 128.65, 128.38, 127.89, 127.74, 127.01, 124.84, 123.97, 123.95, 123.11, 123.07, 65.81, 40.61, 30.38, 29.91, 27.11, 22.87, 20.60, 14.08; HRMS (ESI) calcd for $C_{33}H_{27}N_2O_4$ 515.1965 [M+H$^+$], found 515.1965.

UV-Vis absorption and fluorescence spectra of naphthalimide probes

For **NP-Oxm/Dmf/Str/Nob/BCN/DIBO**, stock solutions of probes were prepared at 10 mM in DMSO. A fresh work solution of corresponding probe was prepared by diluting the stock solution to CH_3CN to make a final concentration of 10 μM. The UV-Visible spectra and fluorescence spectra were recorded using a Tecan Spark™ 10M Multimode Microplate Reader. The fluorescence images of naphthalimide probes in cuvettes were captured as soon as the excited UV lamp turned on.

For **NP-BCN-UV** and **NP-DIBO-UV**, 1 mL of 10 μM **NP-BCN** or **NP-DIBO** in CH_3CN was photoirradiated with a handheld UV lamp (8W, 254nm, 302 nm, or 365 nm) on ice for certain time, then the UV-Visible spectra and fluorescence spectra were recorded using the same method mentioned above.

For the *in situ* fluorescence measurement, 1 μL of 10 mM **NP-Oxm** in DMSO and 1.5 μL of 10 mM **PIFA** in DMSO were added to 992.5 μL CH_3CN. After 1 min, 5 μL of 10 mM BCN or DIBO in DMSO was added to the solution, then incubated at r.t. for 1 h. The solution was photoirradiated with a handheld UV lamp (8W, 365 nm) on ice for certain time (5 min for BCN and 10 min for DIBO), then the UV-Visible spectra and fluorescence spectra were recorded using the same method mentioned above.

DFT calculations

All quantum chemical studies were performed with the Gaussian 09 software

package.[4] Molecular structures for **NP-Iso** and **NP-Oxa** were obtained by optimizing their geometries at the DFT-B3LYP level with 6-311G(d) basis set using IEFPCM model to include solvent (CH_3CN) effect. Vertical excitation energies were computed based on the solvent-equilibrated ground-state geometries using time-dependent DFT (TD-DFT) with the 6-311+G(d) basis set with added diffuse functions. Electron density differences between the ground and respective excited states were determined from the corresponding Gaussian cube output files.

Protein labeling

For preparation of BSA-BCN or BSA-DIBO, a solution of BSA (400 μL, 20 mg/mL) in PBS (pH 7.4) was incubated with a solution of BCN-NHS ester or DIBO-NHS ester (100 μL, 25 mM) in DMSO overnight at room temperature. The excess of BCN/DIBO-NHS ester was removed by spin-filtration (MWCO = 3 kDa). BSA-BCN or BSA-DIBO was then redissolved in PBS (pH 7.4) to a final concentration of 2 mg/mL.

For fluorogenic labeling, 3 μL of 10 mM **NP-Oxm** in DMSO and 4.5 μL of 10 mM **PIFA** in DMSO were added to 32.5 μL CH_3CN. After 1 min, 10 μL of BSA-BCN or BSA-DIBO conjugate (2 mg/mL) was added to the solution, then incubated at r.t. for 1 h. The solvent was removed by freeze drying and the resulting protein was redissolved in 30 μL PBS (pH 7.4) and analysed by SDS-PAGE. For UV-induced fluorescence, the gel was kept on the imager tray and exposed to UV light (365 nm wavelength) for 2 min, then the fluorescence images were recorded. Finally, the gel was subjected to coomassie blue staining.

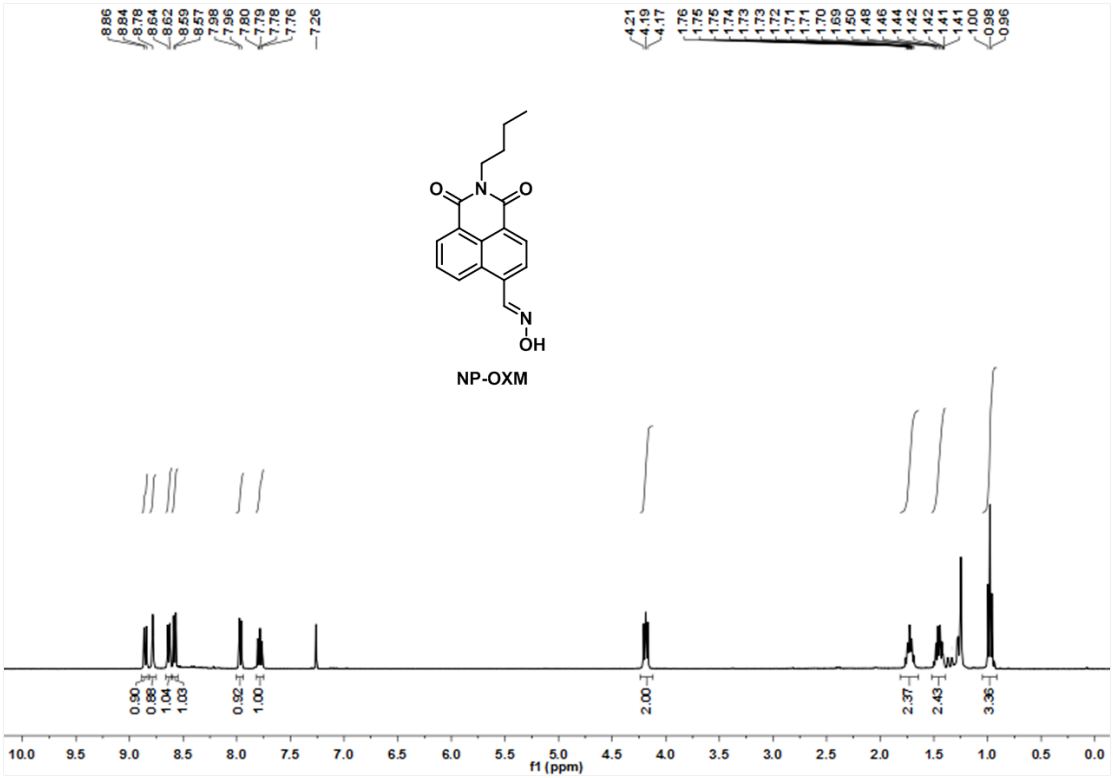
References:

[1] H. Lan, B. Liu, G. Lv, Z. Li, X. Yu, K. Liu, X. Cao, H. Yang, S. Yang and T. Yi, *Sensor. Actuat. B-Chem.*, 2012, **173**, 811-816.

[2] A. M. Jawalekar, E. Reubsaet, F. P. Rutjes and F. L. van Delft, *Chem. Commun.*, 2011, **47**, 3198-3200.

[3] B. C. Sanders, F. Friscourt, P. A. Ledin, N. E. Mbua, S. Arumugam, J. Guo, T. J. Boltje, V. V. Popik and G. J. Boons, *J. Am. Chem. Soc.*, 2011, **133**, 949-957.

[4] Gaussian 09, Revision D.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.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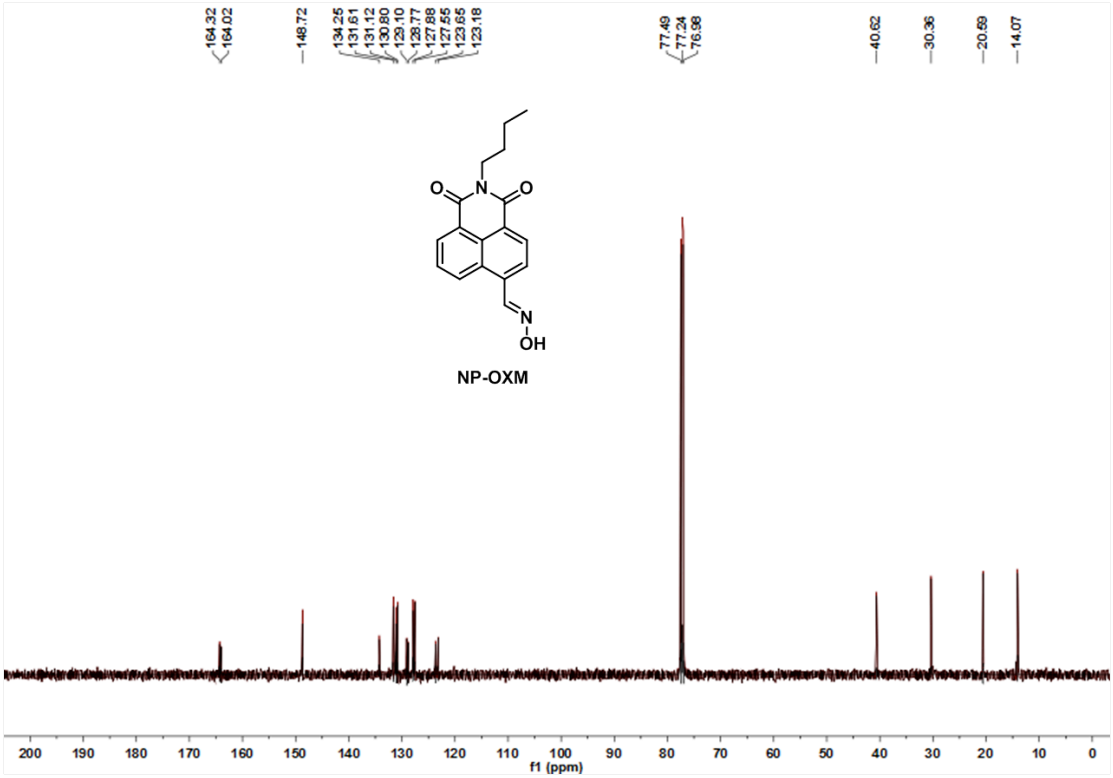
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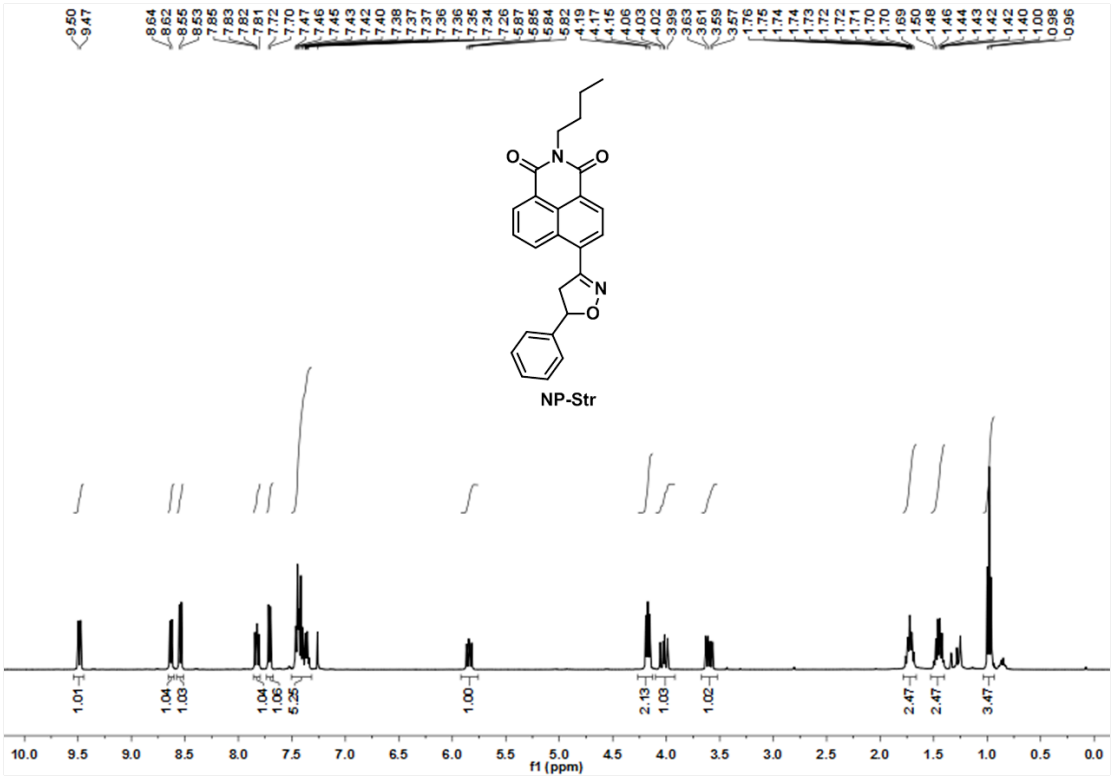
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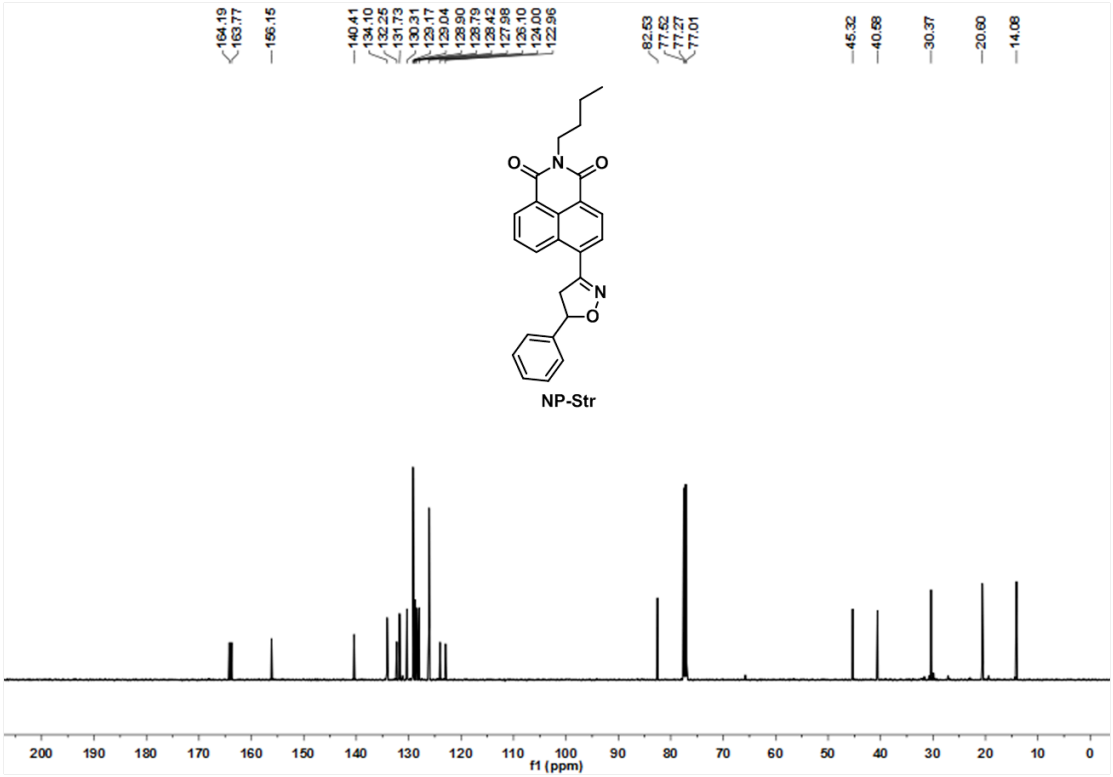
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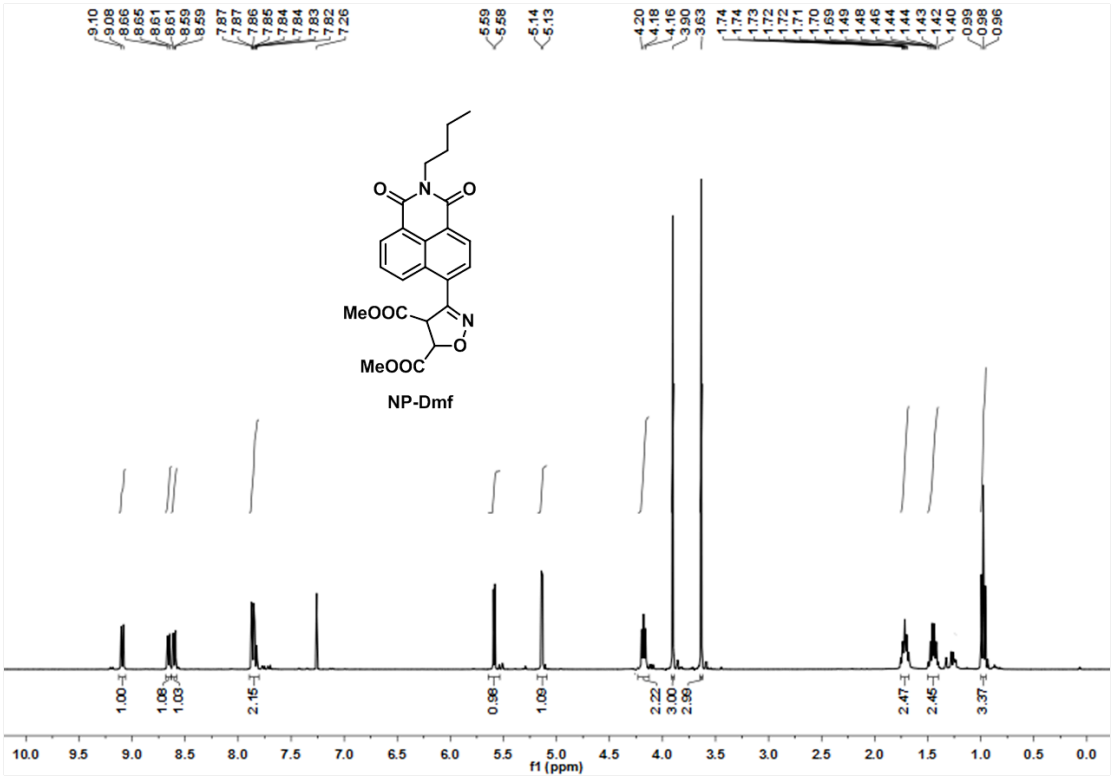
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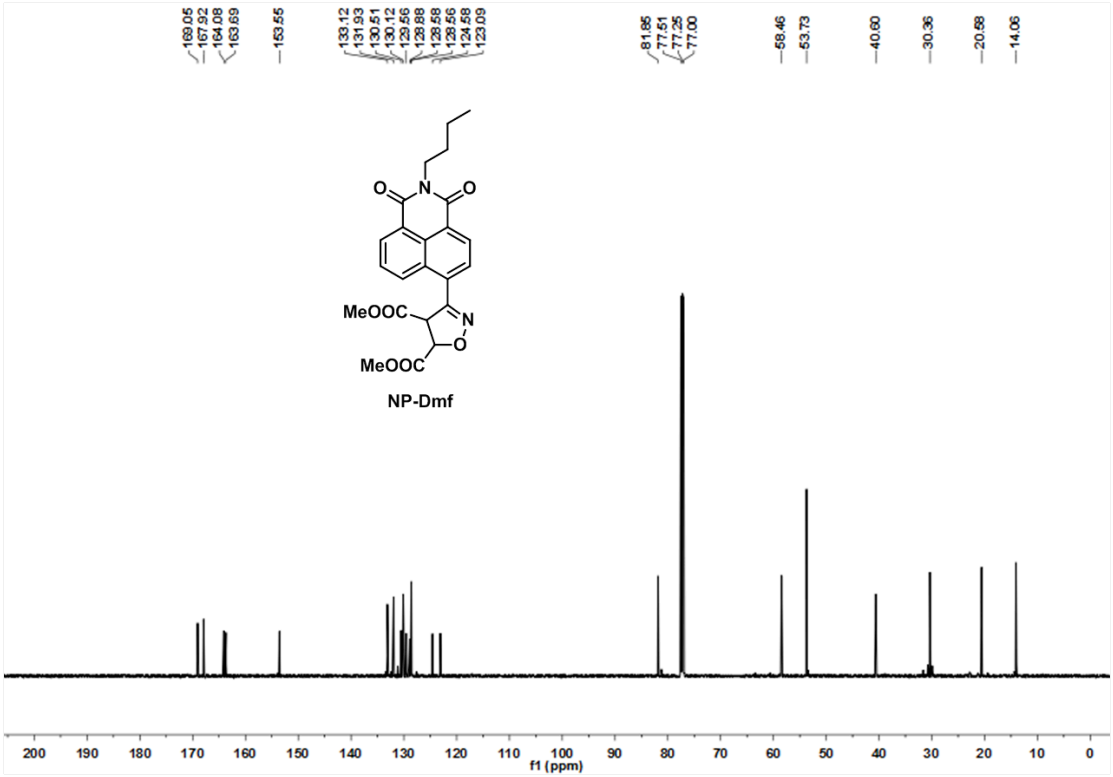
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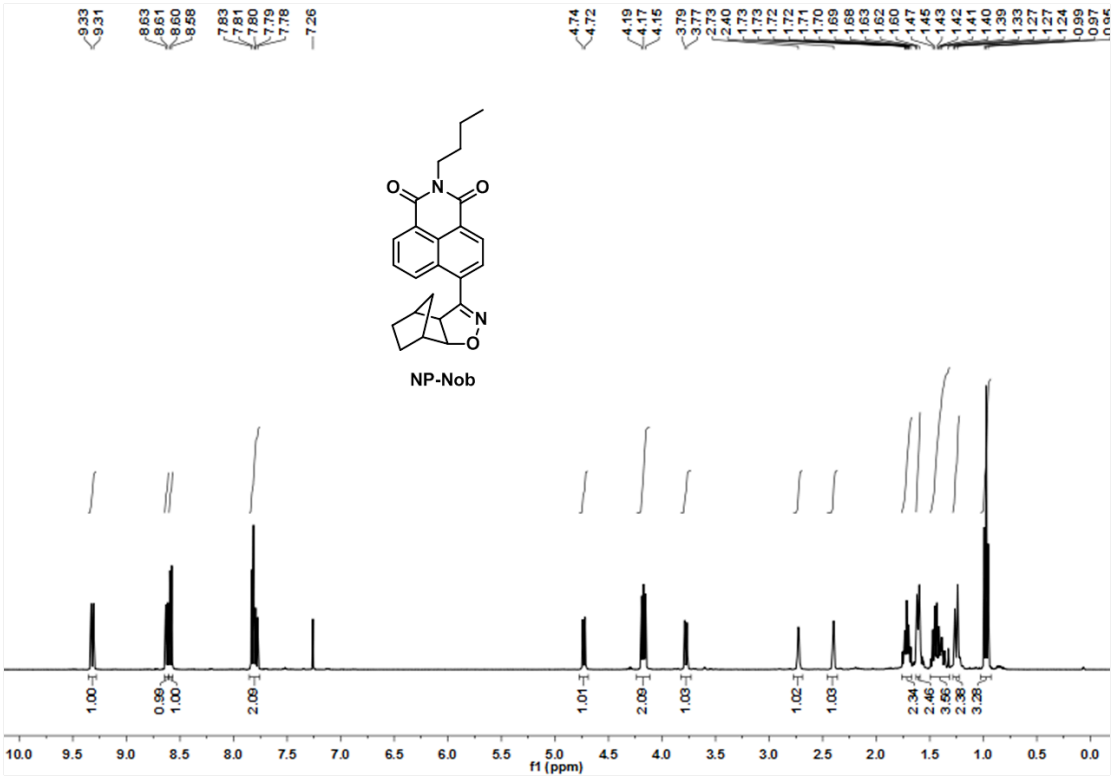
f1 (ppm)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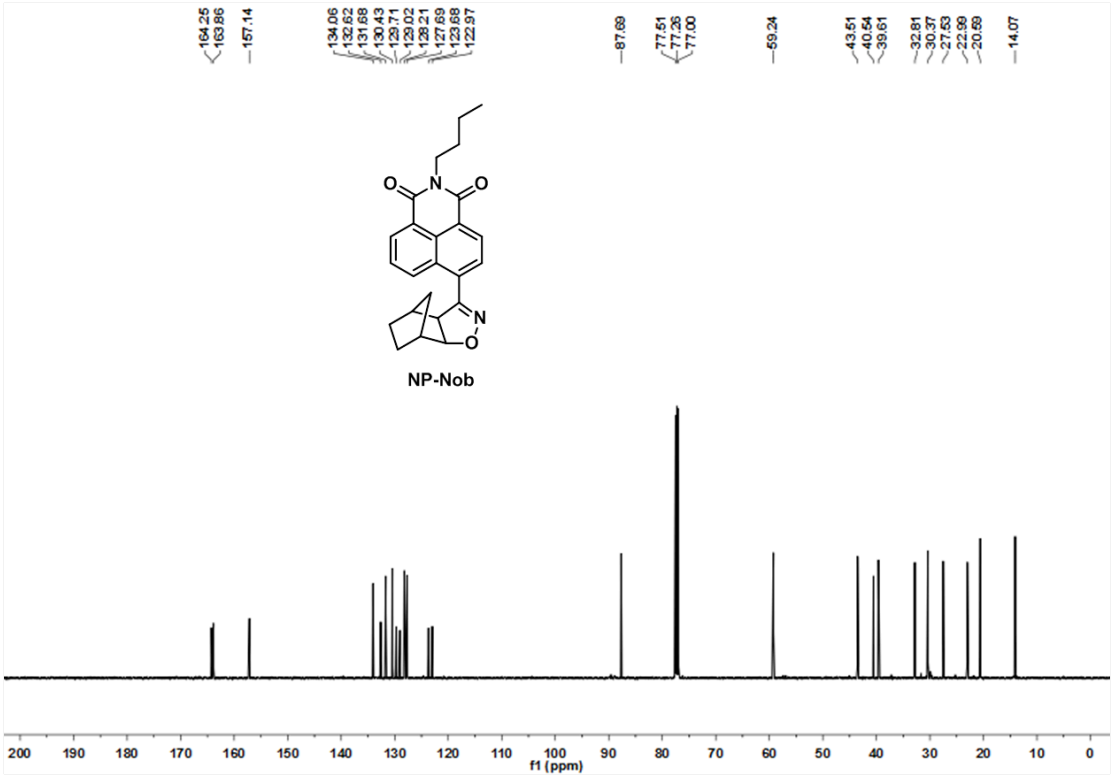
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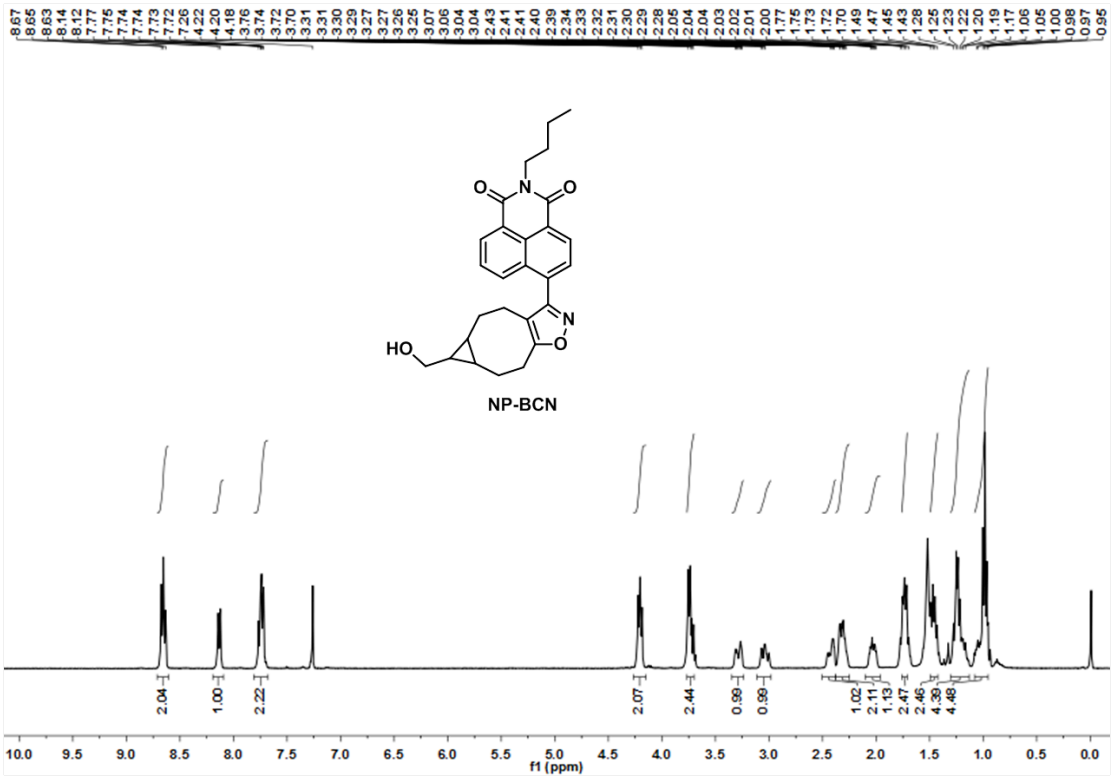
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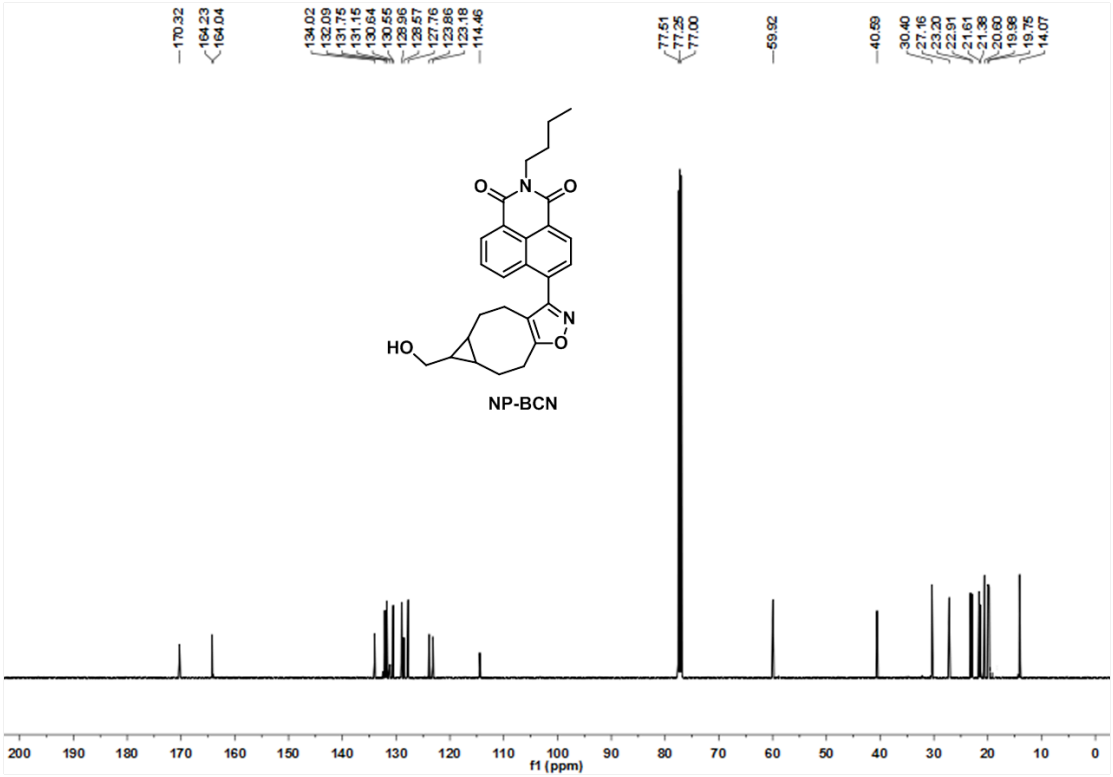
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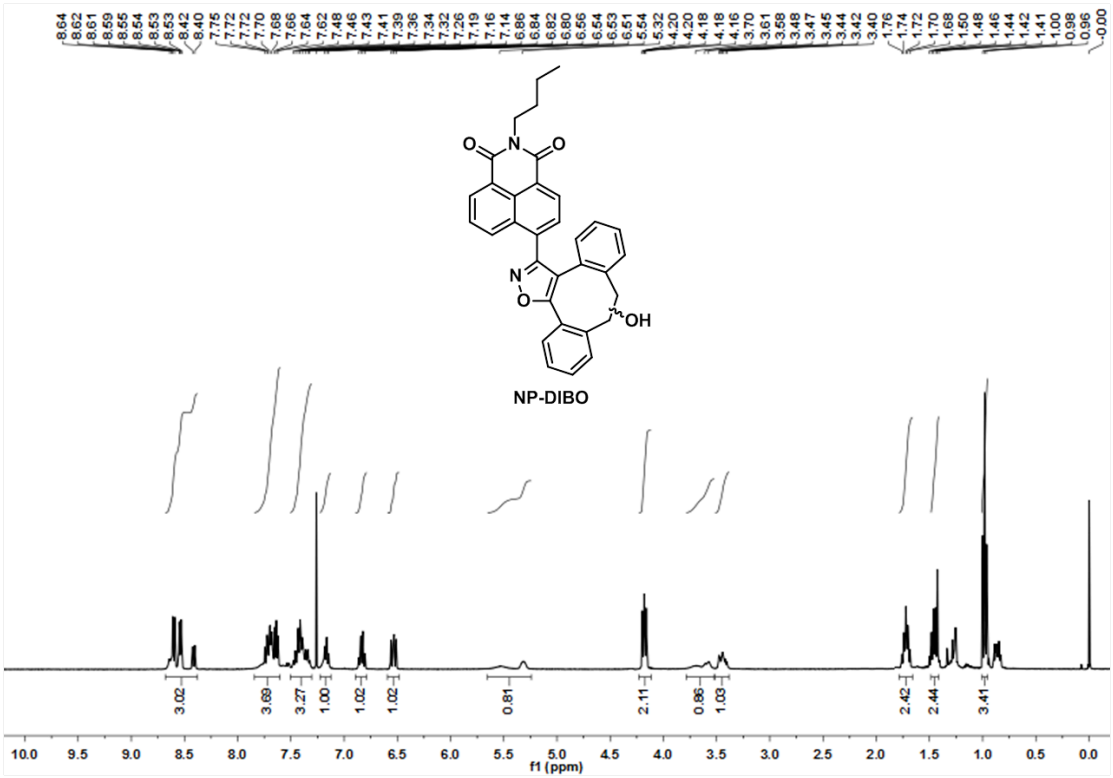
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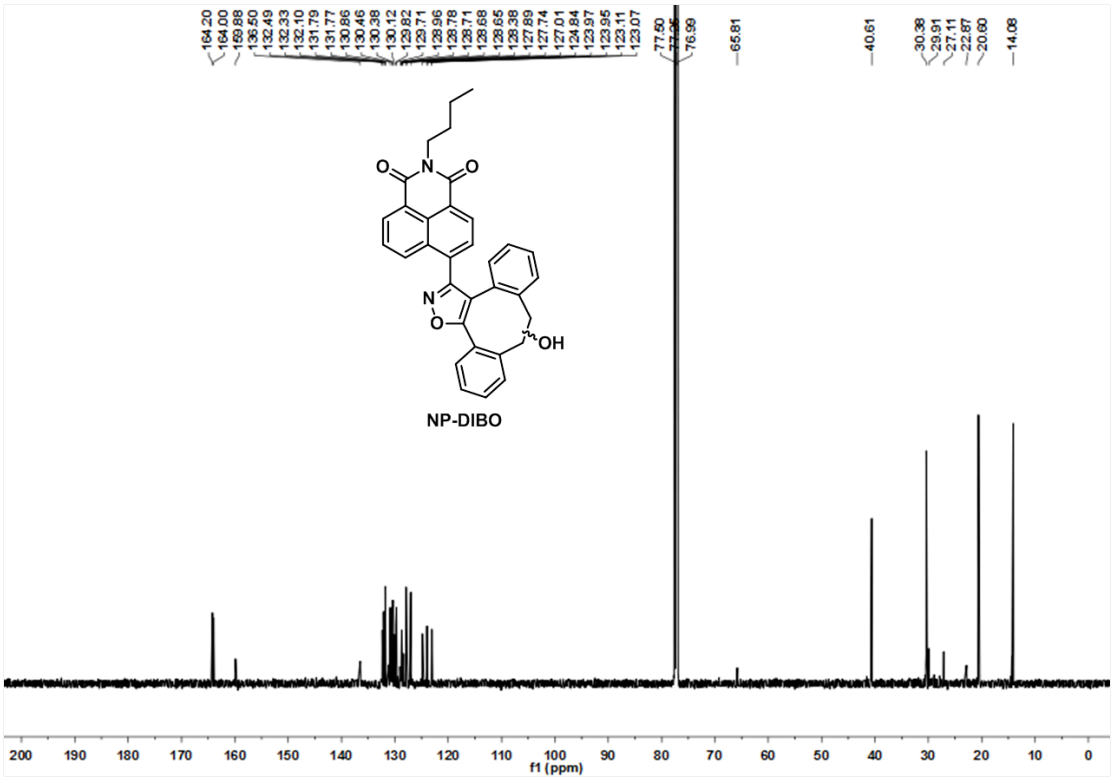
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